# FOOD AND NUTRITION LABORATORY MANUAL

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## LABORATORY MANUAL

DEPARTMENT OF HOUSEHOLD SCIENCE
UNIVERSITY OF ILLINOIS

*Revised Edition*

ISABEL BEVIER, PH.M.
SUSANNAH USHER, S.B.

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Composition and Electrotyping by
THOMAS TODD
14 Beacon Street, Boston, Mass.

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## PREFACE TO SECOND EDITION

In the second edition of this manual the general plan and purpose of the first edition have been retained. The results of later investigations have been utilized in regard to classification and methods of experimentation.

The authors wish to acknowledge the valuable assistance rendered by Miss Helen Isham, Ph.D., in the revision of the experimental work; also their indebtedness to Dr. H. S. Grindley, Professor of Animal Chemistry, and Mr. A. D. Emmett, Research Assistant in Animal Chemistry in the University of Illinois, for valuable suggestions about the work in meat and its extractives.

I. B.
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# LABORATORY MANUAL

## INTRODUCTION

IN the preparation of this laboratory guide the intention has not been to devise new and original experiments, but rather to select from the large body of experiments now offered in physiological chemistry those which in themselves, or in their applications, have a more or less direct bearing upon the principles governing the selection and preparation of food.

The task is a somewhat difficult one, because the greater number of experiments given in text-books of physiological chemistry are designed for students of medicine, and therefore put the emphasis upon the medical phase of the subject.

This manual is intended primarily for the use of students in the Department of Household Science of the University of Illinois. The subject-matter is, therefore, arranged with reference to the correlation of the work of this department with that of the pure science work given in the University. For these students the guide is expected to serve two purposes: first, to aid the student to recall and to arrange in an orderly way the knowledge gained from other sources; second, to apply this knowledge, in so far as possible, to various kinds of food problems, and so to serve as an introduction to individual work with foods which follows.

# FOOD AND NUTRITION

## PROTEIN SUBSTANCES

Among food principles proteins assume first importance, whether we consider their abundance, the variety of forms in which they occur, their part as tissue builders, or their decomposition products. Protein is an essential constituent of the cell, and not one of the phenomena of life is performed without its presence. Different varieties of protein exist in both animal and vegetable organisms.

The complexity of protein substances is shown by the number of compounds obtained from them, and their instability by the ease with which they are broken up into other compounds. Neither the molecular structure nor the chemical formula of protein is known. The elements always found in protein are carbon, hydrogen, nitrogen, and oxygen. Many contain sulphur, a few phosphorus and iron.

The percentage composition of some of the more important members of the group is within the following limits:

| | |
|---|---|
| Carbon | 51.3–55.0 per cent |
| Hydrogen | 6.7– 7.3 per cent |
| Nitrogen | 15.5–19.3 per cent |
| Oxygen | 20.8–23.5 per cent |
| Sulphur | 0.3– 2.2 per cent |

## CLASSIFICATION

Owing to the incompleteness of our knowledge concerning protein substances, little uniformity exists in their classification. The following scheme for classification has been recommended by the joint committees of the American Physiological and Bio-Chemical Societies on protein nomenclature.

### "Recommendations of the Committee on Protein Nomenclature"

### Proteins

"Since a chemical basis for the nomenclature of the proteins is at present not possible, it seemed important to recommend few changes in the names and definitions of generally accepted groups, even though in many cases these are not wholly satisfactory. The recommendations are as follows:

"First. The word *proteid* should be abandoned.

"Second. The word *protein* should designate that group of substances which consists, so far as at present is known, essentially of combinations of *a*-amino-acids and their derivatives; *e. g.*, *a*-amino-acetic acid or glycocoll, *a*-amino-propionic acid or alanine, phenyl-*a*-amino propionic acid or phenylalanine, guanidine-amino-valerianic acid or arginine, etc., and are therefore essentially polypeptides.

"Third. That the following terms be used to designate the various *groups of proteins*":

I. *Simple Proteins.*—Protein substances which yield *only a-amino acids* or their derivatives on hydrolysis.

(*a*) Albumins.—Soluble in pure water and coagulable by heat; *e. g., egg albumin, serum albumin, lactalbumin, vegetable albumins.*

(*b*) Globulins.—Insoluble in pure water, but soluble in neutral solutions of salts of strong bases with strong acids, *e. g., serum globulin, ovoglobulin, edestin, amandin,* and other *vegetable globulins.*

(*c*) Glutelins.—Proteins present in seeds of cereals, and insoluble in *all neutral solvents.*

(*d*) Alcohol soluble proteins.—Proteins soluble in 70 to 80 per cent alcohol, insoluble in water, absolute alcohol, and other neutral solvents, *e. g., zein, gliadin, hordein.*

(*e*) Albuminoids.—Proteins possessing a similar structure to those already mentioned, but characterized by a pronounced insolubility in all neutral solvents, *e. g., elastin, collagen, keratin.*

(*f*) Histones.—Basic proteins which stand between protamines and true proteins, *e. g., globin, thymus histone, scombron,* etc.

(*g*) Protamines.—*The simplest natural proteins,* possessing strong *basic* properties and forming stable salts with strong mineral acids, *e. g., salmin, sturin, clupein, scombrin.*

II. *Conjugated Proteins.*—Substances which contain the protein molecule united to some other molecule or molecules otherwise than as a salt.

(*a*) Nucleoproteins.—Compounds of one or more protein molecules with nucleic acid, *e. g., cytoglobulin, nucleohistone.*

(*b*) Glycoproteins.—Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid, *e. g., mucine* and *mucoids* (*Osseomucoid, tendomucoid, ichthulin, helicoprotein,* etc.).

(*c*) Phosphoproteins.—Compounds of the protein molecule with some, as yet undefined, phosphorus—containing substances other than a nucleic acid or lecithin, *e. g., caseinogen, vitellin,* etc.

(*d*) Haemoglobins.—Compounds of the protein molecule with haematin, or some similar substance, *e. g., haemoglobin, haemocyanin.*

(*e*) Lecithoproteins.—Compounds of the protein molecule with lecithins, *e. g., lecithans, phosphatides.*

III. *Derived Proteins.*

1. *Primary Protein Derivatives.*—Derivatives of the protein molecule apparently formed through hydrolytic changes which involve only slight alteration of the protein molecule.

(*a*) Proteans.—Insoluble products which apparently result from the incipient action of water, very dilute acids, or enzymes, *e. g., myosan, edestan.*

(*b*) Metaproteins.—Products of the further action of acids and alkalis whereby the molecule is so far altered as to form products soluble in very weak acids and alkalis, but insoluble in neutral fluids, *e. g., acid albuminate, alkali albuminate.*

(*c*) Coagulated proteins.—Insoluble products which result from (1) the action of heat on their solutions, or (2) the action of alcohol on the protein.

2. *Secondary Protein Derivatives.*—Products of the further hydrolytic cleavage of the protein molecule.

(*a*) Proteoses.—Soluble in water, non-coagulable by heat, and precipitated by saturating their solutions with ammonium, or zinc sulphate, *e. g., protoproteose, deuteroproteose.*

(*b*) Peptones.—Soluble in water, non-coagulable by heat, but *not precipitated* by saturating their solutions with ammonium sulphate, *e. g., antipeptone, amphopeptone.*

(*c*) Peptides.—Definitely characterized combinations of two or more amino-acids, the *carboxyl group* of one being united with the *amino* group of the other with the elimination of a molecule of water, *e. g., dipeptides, tripeptides, tetrapeptides, pentapeptides,* etc.

For the purposes of this manual the following scheme is deemed sufficient:

I. Proteins.

1. Simple Proteins.

(*a*) Albumins—egg albumin, serum albumin, lacta albumin, and vegetable albumins.

(*b*) Globulins—serum globulin, ovoglobulin, edestin, and other vegetable globulins.

(*c*) Phospho-proteins (nucleo-albumins)—caseinogen and vitellin.

2. Derived Proteins.
   (*a*) Albuminates—acid albuminate and alkali albuminate.
   (*b*) Proteoses (or albumoses) and peptones—protoproteose, heteroproteose, and deuteroproteose; amphopeptone and antipeptone.
   (*c*) Coagulated proteins—fibrin, and the products of heat coagulation, etc.

II. Compound Proteins.

Include compounds of simple proteins with other bodies; for example, with coloring matter, haemoglobin; with carbohydrates, glucoprotein; with nuclein or nucleic acid, nucleoprotein.

III. Albuminoids.

Collagen, elastin, gelatin.

For fuller information on the subject of proteins the following authors may be consulted: Hammarsten, Hawk, Jackson, Long, Mann, Salkowski.

## COMPOSITION OF PROTEINS

Expt. 1. Burn a small piece of dry albumin in a test-tube. Why does it turn black? On continued heating, what is left? Notice the characteristic odor.

Expt. 2. Mix a small amount of dry powdered egg albumin with an excess of soda lime. Put the mixture in a dry test-tube and heat gently. Test the vapors that escape with moist litmus paper. What does this show?

Expt. 3. Place about 5 cc. of dilute NaOH in a test-tube with a small quantity of egg albumin and add

two or three drops of lead acetate. Boil the mixture a few minutes and note the change. What is the dark-colored precipitate?

Expt. 4. Mix some of the dry egg albumin with double its quantity of fusion mixture ($Na_2CO_3 + KNO_3$). Place in a crucible and heat cautiously until the mixture becomes colorless. Dissolve the residue in warm water. Acidify one portion of the solution with HCl and add $BaCl_2$. What is the white precipitate? Write the equation. To another portion add $HNO_3$ and ammonium molybdate solution, warm to 35° C.,[1] and shake. What is the yellow precipitate?

## GENERAL REACTIONS OF PROTEINS

Much has been learned concerning the constitution of protein substances by a study of the decomposition products. This decomposition may be accomplished by oxidation or by hydrolysis. The products thus formed exhibit a great variety of properties due to the presence of different radicals or groups of radicals, so no one reaction is distinctive for all proteins.

Among the most important reactions used for identification are those which give a distinctive color and those obtained by precipitation.

Prepare a 2 per cent solution of white of egg as follows: Put the white of an egg in an evaporating dish, cut with the scissors, and then dilute 20 cc. to 1 liter with

[1] The Centigrade scale is used in all these experiments.

distilled water. Shake thoroughly and filter. Reserve the solution for the following tests:

### Color Reactions

Expt. 5. *Biuret reaction.*—To 5 cc. of the albumin solution add an equal value of NaOH or KOH. Add slowly dilute $CuSO_4$ solution. Notice color.

Expt. 6. *Xanthoproteic reaction.*—To 5 cc. of the solution add an equal volume of concentrated $HNO_3$. Heat until you obtain a yellow precipitate or a yellow solution. Cool and add an excess of $NH_4OH$. Note change of color.

Expt. 7. *Millon's reaction.*—To 5 cc. of the solution add a few drops of Millon's reagent. Heat cautiously.

Expt. 8. *Adamkiewicz's reaction.*—To 2 cc. of concentrated $H_2SO_4$ add about 4 cc. (two volumes) of glacial acetic acid[1] and shake. To the mixture add one drop of the undiluted egg albumin. The liquid changes, slowly on standing, more rapidly when slightly warmed, to a beautiful reddish violet color. This reaction is not given by gelatin. Why? To what are the preceding reactions due?

Report the results obtained with albumin, globulin, proteose, peptone, and gelatin, in tabular form, with the reagents you have used.

[1] This color reaction is due to the presence of glyoxylic acid in the acetic acid. The Hopkins-Cole test modifies the above by using a glyoxylic acid solution in place of the acetic acid prepared as follows: One liter of a saturated solution of oxalic acid is reduced by 60 grams of sodium amalgam and allowed to stand until the evolution of a gas ceases, filtered and diluted with two to three volumes of water.

| | Albumin. | Globulin. | Proteose. | Peptone. | Gelatin. |
|---|---|---|---|---|---|
| Water . . . . . | | | | | |
| Alcohol . . . . . | | | | | |
| Ether . . . . . | | | | | |
| Biuret . . . . . | | | | | |
| Xanthoproteic . . . . | | | | | |
| Millon's . . . . . | | | | | |
| Heat . . . . . | | | | | |
| $HgCl_2$ . . . . . | | | | | |
| $Pb(C_2H_3O_2)_2$ . . . . . | | | | | |
| $AgNO_3$ . . . . . | | | | | |
| $HNO_3$ . . . . . | | | | | |
| $H_2SO_4$ . . . . . | | | | | |
| HCl . . . . . | | | | | |
| $H(C_2H_3O_2)$ strong . . . | | | | | |
| $HC_2H_3O_2 + NaCl$ . . . | | | | | |
| $HC_2H_3O_2 + K_4Fe(Cn)_6$ | | | | | |
| Picric acid . . . . . | | | | | |
| Tannin . . . . . | | | | | |
| $(NH_4)_2SO_4$ (saturated) . | | | | | |
| $MgSO_4$ (½ saturated) . | | | | | |
| NaCl (saturated) . . . | | | | | |
| Dilute salt solution . . | | | | | |

### Precipitation

Expt. 9. To about 3 cc. of white of egg solution add excess of strong alcohol. Does this produce a precipitate? Does alcohol coagulate proteid?

Expt. 10. Dilute 5 cc. of the albumin solution with twice its bulk of 0.1 per cent $H_2SO_4$. Add ether and shake briskly. Note the result.

Expt. 11. To 3 cc. of albumin solution add a drop or two of mercuric chloride. A white precipitate forms. Repeat experiments with lead acetate and silver nitrate.

Why is white of egg given in cases of poisoning with metallic salts?

Expt. 12. Try the effect of the following reagents on egg albumin, using 3–5 cc. of the albumin solution:

$HCl$, $HC_2H_3O_2$ strong,
$H_2SO_4$, $HC_2H_3O_2$ strong + excess $NaCl$,
$HNO_3$.

Expt. 13. To about 5 cc. of albumin solution add one to two drops strong acetic acid and then one to two drops of potassium ferrocyanide. Does this give a precipitate?

Expt. 14. Place 20 cc. of 5 per cent egg albumin solution in a beaker. Add powdered $(NH_4)_2SO_4$ to saturation, and keep in a water-bath at about 35° for half an hour. Stir frequently until the salt ceases to dissolve. Note the formation of a precipitate. Filter and test the filtrate with the biuret test and the precipitate with Millon's test. Explain the results. When

making the biuret test in the presence of $(NH_4)_2SO_4$ or $MgSO_4$ add a large excess of KOH in solid form.

Expt. 15. Place 20 cc. of 5 per cent egg albumin solution in a beaker and add powdered $MgSO_4$ to saturation; digest with frequent stirring at 35° for about half an hour. Observe formation of a precipitate. Filter and test the filtrate and precipitate as in the above experiment, using the same caution in making the biuret test. What protein is present in the filtrate?

### Coagulation by Heat

Expt. 16. Place about 5 cc. undiluted egg albumin in a test-tube with a perforated cork, through which passes a thermometer. The bulb of the thermometer should be immersed in the albumin. Suspend the tube in a beaker of water and heat gradually. Note the temperature at which the albumin clouds. Note the temperature at which it becomes solid. The albumin is heated more evenly if the water is stirred during heating. Try Millon's and Xanthoproteic tests on the coagulated protein.

### Heat and Reagents

Expt. 17. In each of four test-tubes place 5 cc. of the egg albumin solution (1–50). To tubes 1 and 2 add respectively 1 cc. and ½ cc. of a 10 per cent NaCl solution. To the tubes 3 and 4 add respectively one and five drops of a 1 per cent acetic acid solution (1 cc. of glacial acetic diluted to 100 cc.). To a fifth tube containing 5 cc. of egg albumin solution (1–10) add 1 cc.

of 10 per cent NaCl solution. Immerse the five tubes in a bath of boiling water for about five minutes, then examine and note the results. Now add one or two drops of the 1 per cent acetic acid to tubes 1, 2, 5, and to tube 4 add 1 cc. of 10 per cent NaCl, and heat again. What is the result?

### Globulin

See Meat
See Flour

Expt. 18. Extract 20 grams of crushed hemp seed with 100 cc. of 5 per cent solution of NaCl for one-half hour at 60° C. Filter while hot through a paper moistened with 5 per cent NaCl solution, and allow the filtrate to cool slowly. Upon cooling, the globulin separates out in crystalline form.

(*a*) Try two color reactions and two precipitation tests on portions of the filtrate.

(*b*) Test the coagulability of the filtrate.

(*c*) Pour some of the solution drop by drop into a beaker of water.

### Phospho–Proteins (Nucleo–Albumin)

For separation of casein from milk see pages 45, 46, 47.

Expt. 19. (*a*) Apply the Millon and the Xanthoproteic tests to powdered casein.

(*b*) Try its solubility in water and in a weak $Na_2CO_3$ solution.

(*c*) Test some of the casein for phosphorus as follows:

Grind about 0.2 gram of the casein with four times its volume of a mixture made up of equal parts of $Na_2CO_3$ and $KNO_3$, and fuse until the evolution of gas has ceased. Cool, dissolve in a small amount of warm water, acidify with $HNO_3$, and add 5 cc. of ammonium molybdate solution and shake vigorously for five minutes. Precipitation will take place more rapidly from warm solutions, but care must be taken not to heat above 70° C. or $MoO_3$ may be precipitated.

## DERIVED PROTEINS

### Albuminates

Expt. 20. *Acid Albuminate.*—Dilute white of egg with about four volumes of water. To 25 cc. of the solution add 5 cc. of two-tenths per cent HCl and warm on the water-bath for two hours at about 45°. Filter and neutralize the filtrate with dilute NaOH solution, being careful not to get enough to redissolve the precipitate. Dissolve some of the precipitate in a little water to which dilute HCl has been added. Boil some of this solution. Does it coagulate? Add some NaCl. Does this cause precipitation?

Expt. 21. *Alkali Albuminate.*—Put the white of an egg in an evaporating dish and add concentrated KOH, drop by drop, with constant stirring. The mass will gradually assume the consistency of jelly. An excess of KOH dissolves the jelly. Cut the jelly in pieces and wash on a cloth with water. Dissolve some of the jelly in water (sufficient alkali is present to make a weak

alkaline solution) by means of gentle heat. Cool and neutralize with dilute HCl. Does the alkali albuminate precipitate?

### Proteoses

Proteoses and peptones may be prepared from commercial peptones such as Witte's.

Expt. 22. Prepare a 20 per cent solution of the peptone. Heat sufficiently to make the solution complete. Put some of this solution in a test-tube and heat to boiling. Does it coagulate?

Expt. 23. To 10 cc. of the solution in an evaporating dish add 10 cc. of saturated $(NH_4)_2SO_4$ solution. Does a precipitate form? Add about 8 grams of powdered $(NH_4)_2SO_4$, and let it stand in a water-bath at about 25°–32° for one-half hour. Notice the sticky precipitate that adheres to the rod and to the sides of the beaker. Transfer the precipitate to a filter and wash with about 10 cc. of saturated $(NH_4)_2SO_4$ solution.

By means of a glass rod gather up the sticky proteose precipitate and transfer it to about 20 cc. of water in a test-tube. While stirring, heat the liquid carefully, and the proteose dissolves completely.

With this aqueous solution of proteose make the following tests, employing small quantities of the liquid:

(*a*) Biuret test.

(*b*) Precipitation with $HNO_3$.

(*c*) Picric acid.

(*d*) Acetic acid and potassium ferrocyanide.

### Peptones

Expt. 24. To some of the filtrate from Experiment 23 add excess of solid KOH and try the biuret test.

For the preparation of peptone and further tests see pages 63, 64, 71, 72.

## COMPOUND PROTEINS

### Glucoproteins

Expt. 25. *Mucin.*—Obtain saliva by chewing some paraffin to start the flow of the secretion. Filter the saliva and use it in the following experiments.

Expt. 26. Add 60 cc. of saliva to 200 cc. of 95 per cent alcohol. Filter off the precipitated mucin and make the following tests:

(*a*) Try some color-reactions for proteins.

(*b*) Dissolve some of the precipitate in weak NaOH and then add dilute acetic acid, drop by drop. Note results.

(*c*) Boil the remaining precipitate in 25 cc. of 10 per cent (by volume) HCl for two to four hours. The result is much more satisfactory if a condenser is used to prevent evaporation. Cool and render the solution alkaline with concentrated KOH; filter, and then test for reducing substance by Fehling's solution. Let the solution stand, as the reduction may not be evident at first.

### Nucleo–Proteins

In recent years much interest has been shown in this group of the compound proteins, partly because of their relation to the purin bodies.

Nucleo-proteins consist of protein and nucleic acid or nuclein. They are widely distributed in the animal body, and while they occur chiefly in cell nuclei they are also found in the protoplasm.

Particular importance attaches to their decomposition products. On digestion with pepsin hydrochloric the more complex nucleo-proteins are split into protein substance and a simpler nucleo-protein rich in phosphorus, known as nuclein. On further decomposition this nuclein yields nucleic acid, which serves to distinguish this group from phospho-proteins or nucleo-albumins.

On hydrolysis they yield three types of decomposition products:

1. Purin bases—xanthin, guanin, hypoxanthin.
2. Pyrimidin derivatives—mucin, thymin.
3. A carbohydrate group.

As purin bases upon oxidation yield uric acid, the importance of nucleo-proteins is readily understood.

Nucleo-protein may be prepared in the laboratory from the pancreas. See page 70.

## ALBUMINOIDS

### Gelatin

Expt. 27. Use a 2 per cent solution of commercial gelatin, or prepare gelatin from tendon.

Try the following tests:

(*a*) Three color tests.

(*b*) Behavior with $H_2SO_4$, alcohol, tannic acid, and $AgNO_3$.

Expt. 28. Procure a tendon, cut it into small pieces, and to 10 grams add 200 cc. of distilled water and 3 cc. of dilute acetic acid. Boil one-half hour, strain through cheese cloth, and evaporate it on the water-bath to 15 cc. Transfer it to a warm test-tube, insert a thermometer, and note the temperature at which the gelatin solidifies.

### Decomposition of Proteins

In studying the constitution of protein substances they have been separated into simpler compounds. These compounds are known as dissociation or decomposition products. Through the work of E. Fischer and his associates, many of these products have been obtained. Mann groups them as follows:

(*a*) Open-chain amino-acids; (*b*) ring compounds; (*c*) ammonia; (*d*) thio-amino acids. Leucin, glycocoll, and tyrosin are all members of the first group, and at the same time the best known of all the dissociation products. Leucin was the first of these products to be discovered.

For the preparation of tyrosin and leucin see Digestion, page 64.

### Questions

1. Name the forms of protein that you have obtained in the laboratory.

2. Name some common food in which each may be found.

3. What protein substances would you expect to find in the following: raw custard, cooked custard, consommé, beef tea?

4. Have you been able to deduce any principles concerning the cooking of foods rich in proteins?

5. Distinguish between precipitation and coagulation.

6. Give some commercial use of the coagulation of proteins.

7. In what forms may protein be precipitated?

8. Explain the use of fractional coagulation.

9. Why is tannin used in curing hides?

10. Why is white of egg given in cases of lead poisoning? Explain the use of white of egg in the clearing of soups.

11. Is there any reason why vinegar should be used in the preparation of tough meat?

12. How is the setting of gelatin explained?

13. How is this power lost or destroyed?

14. What is the chief use of gelatin in the household?

15. How does it rank among food materials?

# FATS

Chemically considered the fats are glycerides of the fatty acids. They are widely distributed in nature in both plants and animals. When used as food they become an important source of energy for the body, as they are rich in carbon.

*References:* Hammarsten, Allen, Sherman, Thorpe, Leffmann and Beam.

Expt. 29. Compare some of the common fats, such as tallow, lard, and butter, with respect to their color, odor, and taste.

Expt. 30. Place a drop of fat on a piece of paper. Do the same with a drop of ethereal oil and compare the results upon standing.

Expt. 31. Test a small portion of rancid fat by dissolving in alcohol and adding a solution of neutral litmus or red rosalic acid. To what is the rancid odor due?

Expt. 32. Test the solubility of fats in water, alcohol, ether, and chloroform.

Expt. 33. Shake a drop of neutral olive oil with about 5 cc. of water in a test-tube. Repeat, using a very dilute $Na_2CO_3$ solution, a soap solution, and a dilute albumin solution, in place of the water. Compare the permanency of the emulsions so formed. What is an emulsion?

Expt. 34. *Saponification.*—Dissolve 15 grams of KOH in 10 cc. water, add to the solution 100 cc. of 90

per cent alcohol, and heat on the water-bath. Add to this solution 50 grams of lard which has been dissolved by warming in an equal volume of alcohol. Saponification takes place readily on mixing the solutions, and is complete when a drop of the solution dissolves completely in water, leaving no fat globules suspended. The fatty acid may be obtained from this alcoholic soap solution by bringing to acid reaction with dilute $H_2SO_4$ and cooling, when the fatty acid comes to the top and solidifies as a cake, which may be further purified by melting in hot water, and after vigorous stirring cooling to the solidification temperature again.

Save the alcoholic solution for the preparation of glycerol.

### Soap from Fatty Acid and $Na_2CO_3$

Expt. 35. Melt a small amount of the acids prepared from lard in a beaker on the water-bath. Add gradually, with constant stirring, a half-saturated $Na_2CO_3$ solution until all the fatty acid has dissolved. This may take some time; an excess of $Na_2CO_3$ should be avoided. After solution has been accomplished pour a small portion into a test-tube and add an equal volume of cold water. A jelly will form. Immerse another portion in a dish of cold water. Soap is precipitated from solution. Allow the rest to stand and cool slowly.

### Separation of Glycerol

Expt. 36. Filter the solution remaining after precipitation of the fatty acids and make it nearly neutral

with NaOH solution, and then neutralize with $Na_2CO_3$ solution. Evaporate nearly to dryness, first over the free flame and then over the water-bath. Mix the residue with about 50 cc. of 90 per cent alcohol. Let this solution stand for some time, and then filter and evaporate the filtrate on the water-bath. Dissolve the residue in absolute alcohol, so that the mixture makes about 25 cc. Add 25 cc. ether, shake, and let it stand until the next day. Filter and evaporate the filtrate cautiously on the water-bath. The glycerol is obtained as a light yellow sirup. The acrolein test may be tried as follows:

Mix a small amount of the glycerol with some powdered potassium bisulphate and heat the mixture in a dry test-tube. Notice the penetrating odor. Test the fumes with some filter paper moistened with some ammoniacal silver oxide solution. The paper turns black. What does this show?

The ammoniacal silver solution is prepared by adding to 5 cc. of silver nitrate solution one half the volume of NaOH solution and dissolving the precipitation in $NH_4OH$.

Expt. 37. *Melting point of fats.*—Find melting points of butter, lard, beef fat, and mutton tallow according to the method used in work on food analysis or in "Principles and Practice of Agricultural Analysis" (Wiley). To what is the difference in melting point due?

### Lecithin

Expt. 38. *Preparation of egg lecithin.*—Separate the yolks of two eggs from the white. Mix the yolks

with 60 cc. of ether and let stand over night; in the morning add 100 cc. of alcohol and filter the solution. Evaporate the filtrate on the water-bath. Dissolve the residue from the filtrate in 15 cc. of ether; filter, and add 50 cc. of acetone to the filtrate obtained. Filter this through a small filter and save the filtrate for cholesterin. With the lecithin thus obtained perform the following experiments:

(*a*) Add a small piece of lecithin to water; shake; notice cloudy appearance of water. No true solution takes place, but an emulsion is formed which can be filtered unchanged. Notice myelin forms, under microscope.

(*b*) *Tests for phosphorus.*—Fuse one-fourth of the lecithin with a fusion mixture in a porcelain crucible. When well fused allow to cool, dissolve in water, acidify with nitric acid, and add ammonium molybdate. Heat to 50° and shake. Does a yellow precipitate form?

(*c*) *Tests for fatty acid.*—Heat one-third of the lecithin with 20 cc. of sodium alcoholate in a flask for one hour; evaporate the alcohol. Notice the residue. It is soap. Dissolve this in water; boil, and notice the formation of soap bubbles. Make the solution acid and allow it to stand in a warm closet over night. Fatty acids collect at the surface. Cool on ice and the layer of fatty acids may be removed.

(*d*) *Tests for glycerin.*—Fuse a small portion of the lecithin with some potassium bisulphate. Note the peculiar irritating odor of acrolein given off. This shows presence of glycerin. Give the formula for lecithin.

(*e*) *Test for nitrogen.*—Fuse some lecithin in a test-tube with a piece of metallic sodium or soda lime the size of a pea. Notice the smell of ammonia and test with a moist piece of red litmus paper.

### Cholesterin

Expt. 39. *Preparation of cholesterin.*—Evaporate the acetone solution from which the lecithin has been precipitated on the steam-bath. Dissolve the residue in 50 cc. of alcoholic sodium hydroxide (10 per cent) and heat on the steam-bath in a flask until all the alcohol has evaporated. Dissolve the residue in 50 cc. of water, transfer to an evaporating dish, add 10 grams of sodium chloride, and again evaporate to complete dryness. Grind this residue fine in a mortar, dry at 105°, and extract with 30 cc. of cold ether. Filter the ether solution, and crystals of cholesterin remain after evaporation of the ether.

Reactions of cholesterin:

(*a*) Evaporate with nitric acid. A yellow mass is obtained. Add ammonia. What change takes place?

(*b*) Mix in the dry state with strong sulphuric acid. Add chloroform. Note change. Does it change further on exposure to air?

(*c*) Evaporate with a mixture of two volumes of sulphuric acid and one volume of ferric chloride solution. Is there a change of color?

(*d*) Dissolve in chloroform and observe crystals under microscope. Write the formula for cholesterin.

## Questions

1. To what series of fatty acids are the fats related?
2. Give an example of an unsaturated fatty acid and a saturated fatty acid.
3. How do you distinguish oils, fats, and waxes?
4. Why is linseed oil used in paint?
5. What forms of fat have you worked with in your laboratory courses?
6. Name some of the ethereal oils that you have used in the laboratory.
7. Do they all come under one classification in chemistry?
8. How is renovated butter made? oleomargarine?
9. What change takes place when fats become rancid?
10. What is the effect of heat on fats?
11. What makes the fumes of hot fat irritating?
12. What is the effect of cold on fats?
13. What are the qualities to be desired in a fat used for cooking purposes?
14. What are the relative merits of beef suet, mutton tallow, lard, and olive oil for cooking purposes?

# CARBOHYDRATES

This is a name applied to a class of compounds which are especially abundant in the vegetable kingdom. While protein bodies form the larger part of the solids in animal tissue, carbohydrates form the chief part of the plants, and occur in the animal kingdom only in small quantities. Owing to their wide distribution, ease of digestion, and relative cheapness they constitute a very important source of food. Because of their easy oxidation they are one of the principal sources of energy in the animal body. Carbohydrates are compounds of carbon, hydrogen, and oxygen. The statement frequently made that the carbohydrate molecule contains six atoms of carbon or a multiple of six, and that the H and O are in the proportion to form water is not true in all cases. Example, Rhamnose $C_6H_{12}O_5$.

*References:* Hammarsten, Richter-Smith, W. A. Noyes.

The principal divisions of the carbohydrates are:

I. Monosaccharides or Glucoses, $C_6H_{12}O_6$.

II. Disaccharides or Sucroses, $C_{12}H_{22}O_{11}$.

III. Polysaccharides or Amyloses, $(C_6H_{10}O_5)n$.

Monosaccharides are further divided (see table), according to the number of carbon atoms they contain, into trioses, tetroses, pentoses, hexoses, etc. Again, they are classified according to derivation into aldehydes and ketones. Ordinary glucose is an aldehyde; ordinary fructose is a ketone. By oxidation (see table) the aldehydes yield acids; ordinary glucose yields gluconic acid, and, on further oxidation, saccharic acid.

DERIVATION OF CARBOHYDRATES

| Alcohols, primary. | Aldehydes. | Acids. | Acids. | Ketones.[1] |
|---|---|---|---|---|
| $C_5H_{12}O_5$ | $C_5H_{10}O_5$ | $C_5H_{10}O_6$ | $C_5H_8O_7$ | . . |
| $C_5H_7(OH)_5$ | $CH_2OH-(CHOH)_2-CHO$ | $C_4H_5-(OH)_4-COOH$ | $C_3H_3(OH)_3-(COOH)_2$ | . . |
| Arbite | Arabinose | Arabonic | Trioxy-glutaric | . . |
| $C_6H_{14}O_6$ | $C_6H_{12}O_6$ | $C_6H_{12}O_7$ | $C_6H_{10}O_8$ | $C_6H_{12}O_6$ |
| $C_6H_8(OH)_6$ | $CH_2OH-(CHOH)_4-CHO$ | $C_5H_6(OH)_5-COOH$ | $C_4H_4(OH)_4-(COOH)$ | $CH_2OH-(CHOH)_3-CO-CH_2OH$ |
| Sorbite | Dextrose | Gluconic | Saccharic | . . |
| Mannite | Mannose | Mannonic | Manno-saccharic | Levulose |
| Dulcite | Galactose | Galactonic | Mucic | Sorbinose |

[1] Ketones result from oxidation of the secondary alcohols. Their oxidation yields acids poorer in carbon.

DIVISIONS OF CARBOHYDRATES

| | Example. | Preparation and occurrence. |
|---|---|---|
| Monosaccharides | | |
| Trioses $C_3H_6O_3$ | Glycerose | Oxidation of glycerin |
| Tetroses $C_4H_8O_4$ | Erythrose | Oxidation of erythrite which is found in some plants |
| Pentoses $C_5H_{10}O_5$ | Arabinose | Action of acid on cherry gum |
| | Xylose | Action of acid on wood gum |
| | Rhamnose[1] | Decomposition of various glucosides |
| | Fucose | Action of acid on sea weed |
| Hexoses $C_6H_{12}O_6$ | Mannose | Oxidation of mannite which is found in plants |
| | Dextrose | Widely distributed in the vegetable kingdom, as in sweet fruits, honey, etc. |
| | Galactose | Action of dilute acid on milk sugar |
| | Levulose | Found in connection with dextrose in vegetable kingdom |
| | Sorbinose | Found in the juice of service berries |
| Heptose $C_7H_{14}O_7$ | | |
| Octose $C_8H_{16}O_8$ | | |
| Nonose $C_9H_{18}O_9$ | | |
| Disaccharides $C_{12}H_{22}O_{11}$ | Sucrose | Widely distributed in nature |
| | Maltose | Found in germinating cereals |
| | Lactose | Found in the milk of animals |
| Trisaccharides $C_{18}H_{32}O_{16}$ | Raffinose | In sugar beet |
| Polysaccharides $(C_6H_{10}O_5)n$ | Pentosans | Found in plants, wheat bran |
| | Cellulose | In nature as cell wall of plants |
| | Starch | Found in plants as reserve material |
| | Dextrin | By heating starch to 200–210° |
| | Glycogen | Found in the liver |
| | Inulin | Found in the roots of compositæ |
| | Lichinin | Found in lichens |
| | Pectin | Found in fruits and vegetables |

[1] $C_6H_{12}O_5$ $CH_3C_5H_9O_5$

The carbohydrates which have the greatest physiological importance and are most used for food are either hexoses or pentoses. A list of the more common forms, with the place of their occurrence, is in the foregoing table. Later, typical forms of the different classes will be studied.

Expt. 40. Compare dextrose, levulose, cane sugar, lactose, dextrin, and starch as follows:

1. Solubility in cold water.
2. Trommer's test and Fehling's test.
3. Iodine.
4. Molisch's test.
5. Fermentation.

(*a*) *Solubility.*—Take 10 cc. water, add 4 grams of the substance to be examined, and shake thoroughly. When the substance is dissolved add 4 grams more, and so on until no more dissolves.

Dilute the solutions already made to 2 per cent and use them in the following experiments.

(*b*) *Trommer's test.*—To 5 cc. of the solution add about one-half its volume of KOH solution. Shake and add, drop by drop, a dilute solution of cupric sulphate. When a slight permanent precipitate of cupric hydroxide appears, heat and notice if reduction takes place.

(*c*) *Fehling's test.*—Put a small amount of Fehling's solution in a test-tube and add four times its volume of water. Boil, to see that the solution itself does not cause precipitate of cuprous oxide. If the precipitate forms, a new solution must be prepared. Add the solution to be tested a few drops at a time and boil after each addi-

tion. A yellowish or a brownish red precipitate shows that reduction has taken place.

(*d*) *Iodine.*—To 5 cc. of each solution add a drop or two of iodine dissolved in potassium iodide. Note color.

(*e*) *Molisch's test* (*Hawk*).—"Place approximately 5 cc. of concentrated $H_2SO_4$ in a test-tube. Incline the tube, and slowly pour down the inner side of it approximately 5 cc. of the sugar solution to which 2 drops of *a*-naphthol solution (about 15 per cent alcoholic solution) has been added, so that the sugar solution will not mix with the acid. A reddish violet zone is produced at the point of contact."

(*f*) *Fermentation.*—Mash up a compressed yeast cake in 16 cc. of water; place 1 cc. of this homogeneous mixture in each of six fermentation tubes, then add to each fermentation tube 10 cc. of a 5 per cent solution of each of the substances to be tested. Examine after 1½ hours and after 24 hours.

Name the products of fermentation obtained in the above experiment.

### Glucose and Levulose

Use a 2 per cent solution of glucose unless otherwise indicated.

Expt. 41. Place some of the dry glucose in a tube and heat gently over a flame. Note changes. The peculiar odor is that of burnt sugar. Allow the tube to cool, then add water and warm slightly. Explain results.

Expt. 42. To some dry glucose add cold, concen-

trated $H_2SO_4$ and let stand. The liquid remains colorless, or at most is light yellow. Compare with similar experiment under cane sugar. Then gently heat the glucose tube. What is formed?

Expt. 43. To some 95 per cent alcohol in a test-tube add a few cc. of dextrose solution. Compare with experiment under dextrin.

Expt. 44. *Formation of osazones from dextrose.*— In a test-tube prepare a mixture of 5 drops of phenylhydrazin, 10 drops of glacial acetic acid, and 1 cc. of saturated salt solution, and boil for a few minutes. Yellow phenylglucosazone crystals will appear on cooling. Write the equations for the formation of the osazones. For more exact experiments see Sherman.

Expt. 45. Try the effect of dry heat and $H_2SO_4$ on cane sugar (see experiments under dextrose).

Expt. 46. Place 50 cc. of the cane sugar solution in a small beaker, add 6–8 drops of concentrated HCl, and boil for 2–3 minutes. Has the cane sugar been inverted?

Expt. 47. Compare the sweetening power of cane sugar, dextrose, and commercial glucose.

### Lactose

For preparation of lactose see milk.

### Maltose

For formation of osazone see dextrose.

Expt. 48. *Hydrolysis of starch by maltose.*—Make a malt extract by allowing 5 grams of malt to soak in 50 cc. of water for 1½ to 2 hours at room temperature.

This malt extract contains some reducing substances, so in order to prove the formation of a reducing substance by the action of malt on starch it is necessary to proceed as follows:

Add to one Erlenmeyer flask 50 cc. of a 5 per cent solution of starch paste and about 1 cc. of chloroform and mark A. To another Erlenmeyer flask add 50 cc. of water and 1 cc. of chloroform and mark B. Then to each add 10 cc. of the malt extract, cork, and allow to stand until the starch solution gives no color reaction with iodine. This will take two or three days. Prepare two test-tubes containing the same amounts of Fehling's solution (about 5 cc. after dilution) and heat to boiling. To the first add 10 drops of the pure malt solution (flask B) from a pipette, and to the second the same amount of the reaction mixture of malt on starch (flask A). Compare the reducing powers of the two solutions.

## POLYSACCHARIDES

### Starch

Expt. 49. Examine wheat, potato, oat, and corn starch under the microscope. Make drawings.

Expt. 50. Soak dried peas in water over night, then cut thin slices and examine under the microscope.

Expt. 51. Examine with the microscope raw, boiled, baked, and mashed potato. Cut thin slices when possible. Make drawings to represent the difference in appearance.

Expt. 52. Place 100 cc. of water in a beaker and boil it; then add 1 gram of powdered starch and continue boiling for 2–3 minutes, stirring constantly. Reserve for following experiment.

Expt. 53. To about 50 cc. of the starch solution in a beaker add ½ cc. of $H_2SO_4$, cover with a watch-glass, and boil for 15 minutes. Replace the water that may be lost by evaporation. Now place some of the liquid in a tube; render alkaline with sodium or potassium hydroxide. Make Fehling's test. If no reduction takes place continue heating the contents of the beaker for another 15 minutes and test as before. Has inversion taken place?

Expt. 54. Spread one teaspoonful of wheat starch on a tin plate and heat in the oven in which a thermometer is suspended. When the oven temperature reaches 200° take out a small amount of starch and test with iodine on a white plate. Repeat the test for each rise of 10°. How are these changes explained?

Expt. 55. *Gelatinization of starch.*—Put 1 gram of potato starch into 100 cc. of water. Shake well, then distribute this solution equally among ten test-tubes. Put a rubber band around these tubes and place them in a water-bath. Heat gradually to 55° C. Then remove one tube and cool it quickly. Repeat for each rise of 5°. Boil the last tube over the free flame. Place in test-tube rack, let stand, and compare. Look at a drop from each test-tube under the microscope and make drawings.

This experiment may also be tried using 3 grams of potato starch and 100 cc. water. In this case there is

sufficient starch present to form a paste when it is heated to the proper temperature.

Expt. 56. Test for starch, substances in which it is likely to be found as an adulterant or extender.

### Dextrin

Expt. 57. Examine dextrin under the microscope.

Expt. 58. Add tannin to a solution of dextrin. Is a precipitate formed?

Expt. 59. Add alcohol to a solution of dextrin. What happens?

Expt. 60. Obtain dextrin from toasted bread. Is toasted bread thoroughly dextrinized?

Expt. 61. Chop a half pint of oysters as fine as possible. To this material add 500 cc. of boiling water slightly acidulated with acetic acid. Strain the liquid through muslin. This liquid contains, besides glycogen, some proteins and gelatin. To remove the latter, first concentrate to a small volume, then add alternately a few drops of HCl and of potassium mercuric iodide till a precipitate ceases to form. Finally, filter off a little of the liquid and test it with acid and reagent to make sure that all the proteins are precipitated. If this is the case, strain the liquid through muslin, then filter through paper, and to the filtrate add two volumes of alcohol and stir thoroughly. Allow the glycogen to settle, then filter off, wash with dilute alcohol (2 parts alcohol to 1 part water). Finally transfer to a beaker, cover with absolute alcohol, and let stand an hour or more. Then filter off the glycogen, fold the filter, and gently squeeze off

excess of alcohol; finally press between several layers of filter paper till dry. Powder, and save for use later.

(*a*) To some glycogen in a small beaker add 20–30 cc. of water and warm. The glycogen dissolves, forming an opalescent liquid.

(*b*) To a portion of the solution just obtained add a few drops of iodine solution (in potassium iodide). A reddish brown color forms. Then heat the contents of the tube. The color disappears, to reappear on cooling.

(*c*) Boil another portion of the glycogen solution with Fehling's solution. Note the result.

(*d*) To some of the glycogen solution add a few drops of HCl and boil a few minutes. Then cool and neutralize, and test a portion with iodine. Try Fehling's on another portion.

## Cellulose

### Hydrolysis of Cellulose

Expt. 62. To one sheet filter paper add 15 cc. $H_2SO_4$ (50 per cent). To this add gradually enough concentrated $H_2SO_4$ to bring the filter paper into solution. If this $H_2SO_4$ solution is tested with iodine directly, or upon allowing a few drops of water to run onto it, but not get thoroughly mixed with it, a blue starch iodide color results. If the amylocellulose solution is precipitated by dilution with water (three times volume), then iodine added, no color is given. Dilute to about 30 cc. with water and boil one hour. Make the solution alkaline with potassium hydroxide and test with Fehling's solution.

## Pectin

Expt. 63. *Preparation of pectase.*—Pare young carrots and reduce them to a pulp. Express the juice, filter, and save it to add to the solution of pectin.

Expt. 64. *Preparation of pectin.*—Put the residue from the previous experiment into a beaker, cover with water, and boil 15 minutes. Filter. Put 5 cc. of the solution into a test-tube and add a few drops of the liquid containing the pectase. Does the juice form a jelly? How long does it take to form a jelly? Extract pectin from apples and repeat the above experiment. Does acid interfere with the action of pectase? What is the reason for the non-production of jelly in the juice of many fruits?

## Questions

1. What are the legitimate uses of commercial glucose in the home?
2. In what form are the carbohydrates of food absorbed in the body?
3. Where and in what forms do glucose and levulose occur?
4. When cane sugar, lactose, and maltose are hydrolyzed, what monosaccharides are obtained?
5. What changes take place in boiling sugar for fondant?
6. What factors influence the final temperature in making fondant?
7. Why is acid or glucose added in making candy?
8. What is the source of commercial lactose? What is its use?

9. What is the commercial use of maltose?

10. How is dextrin prepared commercially?

11. Do our cooking processes affect cellulose?

12. Where is pectin found?

13. What transformations does pectose undergo in fruits?

14. What transformations does pectin undergo in jelly making?

15. Discuss the problems of jelly making.

16. What principles of starch cookery are you able to deduce from above experiments?

17. How would you separate fructose and glucose from invert sugar?

18. How does the strength of the acid influence the rate of inversion of cane sugar?

19. Compare HCl and $HC_2H_3O_2$ in this respect.

# MILK

*References:* Salkowski.
Blyth (5th ed.).
Sherman.
Richards and Woodman.
Leffmann and Beam.
Conn. Yeasts, Molds, and Bacteria in the Home.
Farmers' Bulletin, No. 74.

Separation into constituents; use of preservatives.

EXPT. 65. Examine a drop of milk under the microscope. Sketch the different sized globules present and measure their diameter.

EXPT. 66. Examine microscopically a drop of skimmed milk. What difference is observed between this and whole milk?

EXPT. 67. Boil about 25 cc. of milk in a small beaker for 5 minutes. No coagulation proper, but scum may form. Remove the scum with a spoon or spatula and heat again; a new scum forms. This formation of scum will repeatedly take place. What is the nature of this scum?

EXPT. 68. To about 10 cc. of milk in a test-tube add one drop of dilute acetic acid (1–10), then boil. The casein is coagulated and carries down with it the fat. The serum is clear.

Expt. 69. Place 10 cc. of milk in each of five test-tubes. To No. 1 add ½ cc. of very dilute HCl (10 drops of HCl to 50 cc. of water). To No. 2 add ½ cc. of 2 per cent $Na_2CO_3$ solution. To No. 3 add ¼ cc. of saturated $(NH_4)_2C_2O_4$ solution (1–20). Then add to each of these three tubes, and also to Nos. 4 and 5, 2 drops of rennet solution, and mix. Heat the contents of tube No. 5 to boiling. Then place all the tubes in a water-bath at 40° and examine every 3–5 minutes and explain results. What can you say of the action of alkali on rennet? of acid?

What is the composition of coagulum? What is the clear liquid that separates from the coagulum on standing? Why does not tube No. 3 coagulate? Continue heating tube No. 3 at 40° for about one-half hour, then add 2–3 drops of $CaCl_2$ solution. The liquid instantly solidifies. Why?

Expt. 70. To some milk in a test-tube add 1–2 volumes of ether, close, and shake thoroughly. Do the fat globules dissolve? Now add a few drops of NaOH and shake again. Observe and explain results. This reaction was taken at one time to indicate that the globules were surrounded by an albuminous envelope.

Expt. 71. To some milk in a test-tube add a few drops of NaOH and heat. Result?

Expt. 72. To about 10 cc. of milk in a test-tube add 5 grams of powdered $MgSO_4$ and shake thoroughly. Then pour onto a filter, resting in a test-tube, and set aside to filter over night. Boil the clear filtrate. Result? The casein is precipitated almost completely by $MgSO_4$.

EXPT. 73. To 5 cc. of milk add 4 volumes (20 cc.) of strong alcohol; shake thoroughly and set aside. All the proteins present are precipitated.

EXPT. 74. Add 50 cc. of milk to about 125 cc. of water, mix well, and while stirring add dilute acetic acid (1–10), drop by drop, till the precipitate becomes coarsely flocculent and ceases to increase. Stir thoroughly and set aside over night. Do not add more acid than is necessary. What does this precipitate contain? Filter off the precipitate and allow to drain well, then fold over half the filter in the funnel and apply gentle pressure with the fingers until no more water can be squeezed out.

Transfer the precipitate to a small, dry beaker, add about 30 cc. of strong alcohol, and stir thoroughly so as to dehydrate the caseinogen. Then filter and again squeeze the contents of the filter as dry as possible. Transfer the precipitate to a small, dry beaker; add about 50 cc. of ether and stand over night. Filter, wash with ether, and squeeze as dry as possible.

Spread open the filter on the table; allow the remaining ether to evaporate, then powder. The white, chalky powder is caseinogen.

(*a*) The ether filtrate received in a small beaker or evaporating dish and evaporated cautiously on the water-bath gives the milk-fat.

(*b*) The aqueous filtrate from the casein and fat precipitate contains albumin and milk sugar. Place it in a beaker and boil for 15 minutes, in order to coagulate the albumin. Filter, and treat the filtrate as follows: Heat on a wire gauze over flame until it becomes cloudy

and bumps. If the liquid is cooled the cloudiness disappears. Why? Heat again to boiling and filter hot. Concentrate the filtrate on the water-bath, and if the cloudiness continues to appear filter several times more. When the filtrate is reduced to a sirupy consistency, set it aside until the next day. Crystals of milk sugar should separate on standing.

### Preservatives

*References:* Leffmann and Beam.
Leach.

Expt. 75. Test milk for (*a*) formaldehyde, (*b*) salicylic acid, (*c*) boric acid, (*d*) $Na_2CO_3$ or $NaHCO_3$.

### Questions

1. What is the problem for the housewife in the use of soda and sour milk?
2. Explain the curdling of milk soups.
3. What are the sources of danger in ice cream?
4. Explain the Babcock test used in analysis of milk.
5. What is the use of sucrate of lime in the dairy industry?
6. Discuss alkaline fermentation of milk.

## MEAT

*References:* Mitchell, C. A. Chapters I, VIII, IX, X.

Grindley and Sprague. A Precise Method of Roasting Beef. The University of Illinois Studies, Vol. II, No. 4.

Grindley, H. S. Journal of Amer. Chem. Soc., Vol. XXVI, No. 9, September, 1904. (Nitrogenous constituents.)

Grindley and Emmett. Journal of Amer. Chem. Soc., Vol. XXVII, No. 6, June, 1905; Vol. XXVIII, No. 1, January, 1906. (Analysis.)

Emmett and Grindley. The Chemistry of Flesh.

Trowbridge and Grindley. Chemistry of Flesh. Journal of Amer. Chem. Soc., April, 1906.

Grindley and Associates. Bulletins Nos. 102, 141, 162, 193, U. S. Dept. of Agriculture, Office of Experiment Stations. (Cooking and digestion.)

Mann, Gustav. Chemistry of the Proteids.

Bevier and Van Meter. Selection and Preparation of Food.

Expt. 76. Put 25 grams of finely chopped meat in 75 cc. of water and allow it to stand for one or two hours. Stir frequently. Strain through cheese cloth,

then filter through paper. Reserve the residue for the next experiment. Test the reaction of the solution with litmus paper.

Slowly heat a portion of the filtrate in a test-tube in which a thermometer is placed. Put the test-tube in a beaker half filled with water and heat the beaker on the wire gauze. By frequent stirring of the water with a glass rod, on the under end of which is a piece of rubber tubing, the even distribution of the heat may be accomplished. Even at a slight elevation of temperature, usually at 45°–56°, coagulation occurs. Filter. Heat the filtrate again to 65° and observe results. Filter. Heat to 75° and filter. Make two color tests for protein with the filtrates. Boil some of the original filtrate, filter, and divide into three portions.

Test one portion with Millon's reagent; to another portion add 1 cc. of a 5 per cent NaCl solution and 5 cc. of a 20 per cent tannic acid solution, drop by drop. Observe precipitation. To another portion add a few crystals of $NH_4NO_3$ and 10 cc. of a neutral ammonium molybdate solution. Heat to 60° C. This should precipitate the inorganic phosphates.[1]

Expt. 77. Digest the meat residue from the previous experiment in 100 cc. of a 15 per cent $NH_4Cl$ solution for 24 hours in a covered beaker, then filter and test the filtrate as follows:

(*a*) Pour a part of the solution into a test-tube two-thirds full of water. Myosin forms as a cloudlike precipitate.

[1] The molybdate solution should have for every 50 cc. of the reagent 4 cc. of $HNO_3$ (1.2 sp. gr.).

(*b*) Heat some of the solution in a test-tube. Does it coagulate?

(*c*) Try two color tests. How does this protein differ from the one obtained in the first experiment? Give the names of the proteins obtained from meat.

Expt. 78. Influence of cooking upon the composition of meat.

(*a*) Boil meat under known conditions.

1. Examine cooked meat. Repeat experiments under raw meat.

2. Examine broths. Concentrate some of the broth and make protein tests. Take 200 cc. of the original broth, add 15 cc. of a 10 per cent HCl solution. Evaporate to 25 cc., filter, test for creatinin by Jaffe's reaction.

(*b*) Roast meat under known conditions.

1. Examine roasted meat. Repeat experiments under raw meat.

2. Examine drippings for proteins.

### Extractives

*References:* Grindley, H. S., and Woods, H. S. Creatinin. Jour. Biol. Chem., Vol. II, p. 309.
Emmett, A. D., and Grindley, H. S. Creatinin. Jour. Biol. Chem., Vol. III, p. 491.
Hammarsten.
Salkowski.

Expt. 79. *Creatin and creatinin.*—Extract 500 grams chopped beef with 500 cc. water for half an hour over the water-bath at 50°. Strain as dry as possible through muslin and make a second extraction

in the same way, with an equal amount of water (save residue). Unite the two extracts, and after concentration to about 200 cc. (test 20 cc. for creatinin by Jaffe's reaction; also test Liebig's extract in the same manner) acidify the solution with 2 or 3 drops of acetic acid. Remove the coagulated proteins by filtration, and to the filtrate (this filtrate corresponds to Liebig's extract, and the extract may be used for study instead of the chopped meat) add basic lead acetate, *carefully avoiding any excess;* the precipitate consists of phosphates, chlorides, sulphates, etc. Allow this to settle and then filter. Warm the filtrate and pass $H_2S$ through it to remove the excess of lead. Filter hot. Concentrate the clear filtrate on a water-bath to a thin sirup and allow to stand in a cool place. Crystals of creatin will deposit. Filter off the crystals, and wash them with 88 per cent alcohol.

Place some of the crystals in a small flask with 10 cc. dilute $H_2SO_4$, and heat for half an hour on the water-bath, keeping the volume constant. While still warm add powdered $BaCO_3$ to neutralization. Filter, and evaporate the filtrate to 10 cc. The creatin has been changed to creatinin. Write the equation. Perform the following tests with the solution:

(*a*) Place 2 drops of the solution upon a watch-glass and add to it a few drops of an alcoholic solution of $ZnCl_2$; allow it to stand and then examine the crystals under the microscope.

(*b*) *Weyl's reaction.*—To 2 cc. of the creatinin solution add 3 drops of a freshly prepared dilute solu-

tion of sodium nitroprusside. Then add, drop by drop, dilute NaOH. A ruby-red color is produced, which quickly changes to yellow. If the solution is now acidified with acetic acid and heated, a green color is obtained, and upon continued boiling a precipitate of Prussian blue settles out.

(*c*) *Jaffe's reaction.*—Treat 5 cc. of the solution with a saturated solution of picric acid, and make it alkaline with 1 cc. of a 10 per cent NaOH. The solution immediately becomes a deep red.

Expt. 80. *Xanthine and hypoxanthine* (*Salkowski*). —"Dissolve 50 grams of meat extract in 500 cc. of water in a flask, and after the addition of 75–100 cc. of nitric acid (1.2 sp. gr.), to destroy substances which hinder the precipitation of the xanthine bases by silver nitrate, heat on the sand-bath until the solution has cleared up, which will require about three-quarters of an hour. After cooling make strongly alkaline with ammonia, filter from the phosphates which separate, and add an ammoniacal solution of 2.5 grams of silver nitrate in about 100 cc. of water. The precipitate, which consists for the most part of hypoxanthine silver, besides a little xanthine silver, is then collected on a filter and washed a few times with water.

"The separation of these two xanthine bases is accomplished by converting them into the silver nitrate compounds. These compounds conduct themselves differently towards nitric acid. The hypoxanthine silver nitrate compound is very difficultly soluble in nitric acid; the xanthine silver nitrate compound is far more readily soluble. The following is the best method of procedure:

"Put the still moist precipitate into a flask and pour over it a mixture of 100 cc. of nitric acid and 100 cc. of water; add 1 gram of urea, heat just to boiling, and let cool. The hypoxanthine silver contained in the precipitate is converted into hypoxanthine silver nitrate, which remains partly undissolved and partly goes into solution, but separates out of the solution again on cooling. The addition of the urea is to prevent the formation of nitrous acid, which might decompose the xanthine bases. Filter the hypoxanthine silver nitrate off after a few hours, and wash until the wash water no longer reacts strongly acid. Let the filtrate (without the wash water) stand till next day and filter (without working up the precipitate, which is a mixture of hypoxanthine silver nitrate and xanthine silver nitrate). The filtrate is used to show the presence of xanthine. The hypoxanthine silver nitrate is examined under the microscope (fine needles frequently grouped in the form of stars).

"(*a*) Pour upon a small portion of the substance on a porcelain crucible cover a few drops of strong or fuming nitric acid, and evaporate cautiously to dryness over a small flame. A lemon-yellow residue results, which takes on an orange color when moistened, after cooling, with caustic soda solution. If a drop of water be then added, a yellow solution results, and this when evaporated again leaves an orange residue (distinction from the murexide reaction for uric acid).

"(*b*) Pour upon a small portion of the substance in a dish a little pure nitric acid of the specific gravity 1.2, and evaporate on the water-bath to dryness. The

residue is scarcely perceptibly colored. On the addition of caustic soda it becomes pale yellow (distinction from xanthine and guanine, which under these conditions give the xanthine reaction). Hypoxanthine is further distinguished by its solubility in ammonia and the insolubility of its compound with silver nitrate in nitric acid, and also by the crystal form of this compound.

"(*c*) The filtrate from hypoxanthine silver nitrate contains xanthine, as previously stated, but only in small quantity. Make it alkaline with ammonia (or, in order to save ammonia, neutralize the greater part of the acid with soda or lime and then make alkaline with ammonia). Xanthine silver precipitates in brown or reddish flakes. These are filtered off, washed, suspended in water, some drops of ammonia added, heated, treated with a few drops of ammonium sulphide, shaken thoroughly, filtered from the silver sulphide, and evaporated (or the precipitate may also be decomposed with hydrochloric acid and xanthine hydrochloride obtained on evaporation). Very frequently the silver sulphide passes through the filter; we then evaporate to dryness and extract the residue with boiling water. The xanthine thus obtained is usually not quite pure and the quantity is very small. It suffices, however, for the xanthine test as well as for the so-called Weidel's reaction.

"(*d*) *Xanthine test.*—Dissolve the residue or half of it in nitric acid and evaporate cautiously to dryness on a crucible cover over a small flame. A lemon-yellow residue results, which becomes intensely red on moistening with caustic soda, and on further heating purplish

red. Add a few drops of water and warm; a yellow solution results, which again gives a red residue on evaporation (distinction from the murexide reaction for uric acid).

"(*e*) *The so-called Weidel's reaction.*—Dissolve half of the xanthine obtained in bromine water, warming gently, evaporate the solution on the water-bath to dryness, and invert the dish over another which contains some ammonia. The residue becomes red."

### Questions

1. In what ways is salt used in the preparation of meat?
2. If you wish rare roast beef, what should be the maximum interior temperature of the roast?
3. What are the factors in producing flavor in preparation of meat?
4. What inferences concerning making of soup do you draw from Dr. Grindley's investigations in meat?
5. Discuss the subject of basting of meats.
6. What protein substances are drawn out in cold water extracts of meat?
7. Do the substances extracted by hot and cold water differ in character or quantity? What is the chief loss in cooking meat? How is the percentage of loss of water influenced by method of cooking?
8. Discuss in a general way the changes which take place in meat during cold storage.

*References:* Jago.
Allen.
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EXPT. 81. Examine wheat kernel. Examine sections of wheat kernel under microscope and make drawings.

EXPT. 82. Review physical tests of flour which you have made with low grade and high grade flours from winter and spring wheat.

EXPT. 83. Make "doughing tests" with three different kinds of flour as follows: Mix in an evaporating dish with a glass rod or spatula 45 grams of flour and a measured quantity of water, enough to make stiff dough. Repeat the experiment with the other flours, using water enough to make doughs of the same con-

sistency as No. 1. Observe the cubic centimeters of water used in each case and estimate the amount of water to "dough" 1 pound of each of the flours.

Expt. 84. *Preparation of gluten.*—45 grams flour; water to make a stiff dough. Let it stand one hour. Put in cheese cloth and wash in stream of water. For large amount of flour (as 1 pound) better to lay cloth over sieve and wash. The gluten remains on the cheese cloth. If gluten is to be baked, put in oven immediately.

(*a*) Treat a small portion of this gluten with 10 per cent sodium chloride solution. Does it dissolve the gluten? Grind some of the gluten in a mortar with alcohol (70 per cent by volume). Filter. Observe change in residue. Explain it. Test the filtrate as follows: To one portion add distilled water. Boil one portion. Make biuret test on another portion. What proteins have you obtained?

(*b*) Test two flours in the aleurometer (see Allen). For further work in separation of proteins of wheat flour see Osborne.

### Questions

1. Compare the flours obtained from spring and winter wheats, giving characteristics of each kind.
2. What difference in manipulation do these flours require in the making of bread?
3. Describe the changes which the different food principles undergo in the making of bread.
4. Discuss the factors that produce sour bread.
5. What qualities of bread are affected by the stage at which fermentation is arrested?

6. What are the characteristics of a good macaroni flour?

7. Discuss American macaroni.

8. Describe the general structure of the wheat grain.

9. From what part of the grain is the larger part of the flour obtained?

10. What do you understand by the term "aleurone layer"?

11. Do "long"and "short" process bread differ in flavor, texture, or chemical composition?

12. How can you distinguish gliadin and glutenin?

13. In what way do these substances influence the bread-making qualities of a flour?

14. Upon what do the bread-making qualities of a flour depend?

15. Upon what does the nutritive value of a flour depend?

## DIGESTION OF STARCH

Expt. 85. Prepare an iodine solution. Make a starch paste by boiling 30 grams of starch in 500 cc. of water. Boil until it becomes translucent, stirring thoroughly. Put 25 cc. of this paste into each of six bottles and stand five of them on the water-bath, to be kept at a temperature of 40° C.

Number the bottles 1, 2, 3, 4, 5, and 6, respectively. Place No. 5 in ice water. Weigh out carefully 1 decigram of pepsin for No. 1, 1 decigram of pancreatin for No. 2, 1 decigram of amylopsin each for 3 and 5. Put a small amount of saliva in No. 6.

Put these weighed quantities of the ferments into their respective bottles, and shake thoroughly. Notice that (4) is only for comparison.

How does the ferment in each case affect the starch mass? In which does it liquefy most? Take five ordinary test-tubes and fill with water. Set them in a row, or number with a gummed label. Drop one small drop of iodine solution into each by means of a pipette, and shake to mix.

When the enzymes have been at work for five minutes, take a clean pipette and drop one drop of No. 1 into the first tube of iodine solution and put one drop of No. 2 into second iodine tube, and so on for each tube. Rinse the pipette each time. Then shake the iodine solutions. Test tube (6) every few seconds by

adding a drop of solution from (6) to a drop of iodine on a white plate.

No. 4 will show what pure starch and iodine will give. How do the others compare? In which do you find the least change of color of the iodine? Allow to stand 5 minutes more and repeat the same, having replenished your test-tubes with iodine solution. What is the effect of temperature as shown by the ice water?

After you have settled the question as to which of these ferments is most active in digesting starch, test the product of digestion to determine what change has taken place in the starch. Compare the action of saliva on starch paste and raw starch. What do you conclude?

# DIGESTION OF PROTEINS

EXPT. 86. Fibrin may be used in the following experiments instead of white of egg, also natural instead of artificial gastric and pancreatic juices.

*References:* Howell.
Hammarsten.
Salkowski.
Grindley. Bulletin No. 193, U. S. Dept. of Agriculture, Office of Experiment Stations.

I. Make up solutions as follows:

1. Dilute 6 cc. HCl (1.19 sp. gr.) to 1 liter.
2. Add to solution (1) 1 gram pepsin (or 2 grams).
3. Add to 1,000 cc. water 1 gram pepsin.
4. Add to 1,000 cc. 1 per cent $Na_2CO_3$, 1 gram pepsin.
5. 1,000 cc. 1 per cent $Na_2CO_3$ + 2 to 3 grams pancreatin.
6. 2 to 3 grams pancreatin to 1 liter solution (1).
7. 2 grams amylopsin + 1,000 cc. 1 per cent $Na_2CO_3$.

II. Prepare test-tubes or bottles as follows:

Boil an egg hard and put the white through sieve. Use in following experiments:

(*a*) In tube (*a*) put ½ gram white of egg and 20 cc. solution (2).

(*b*) ½ gram white of egg in one piece + 20 cc. solution (2).

(*c*) ½ gram white of egg in 20 cc. solution (1).

(*d*) ½ gram white of egg in 20 cc. solution (3).

(*e*) ½ gram white of egg in 20 cc. solution (4).

(*f*) ½ gram white of egg in 20 cc. solution (5) and thymol.

(*g*) ½ gram white of egg in 20 cc. solution (6).

(*h*) ½ gram white of egg in 20 cc. solution (7).

(*i*) ½ gram yolk of egg in 20 cc. solution (5).

(*j*) Prepare a second tube like (*a*) and put in a cool place.

(*k*) Prepare a tube like (2) and add a few shreds of muscle fiber washed until white.

(*l*) Prepare a tube like (*i*) and add washed muscle fiber.

(*m*) Prepare a fifth tube containing 20 cc. solution (5) and add washed muscle fiber.

Place tubes thus prepared and labeled in warm oven at 40° C. Let the materials digest for 2 or 3 days and note carefully the results. At the end of 2 or 3 days test the contents of the tube for the products of digestion.

### Products of Protein Digestion

(*a*) *Peptic digestion.*—Dilute 3 cc. of concentrated HCl to 500 cc. with distilled water, and add ½ gram of soluble pepsin. Add this solution to the whites of four boiled eggs rubbed through a sieve. Allow to stand in a warm place, preferably at 40° C., and not above 56° C., with frequent shaking. After four days filter, and test a small portion of the solution for acid albuminates by just neutralizing with NaOH; filter out

the acid albuminate and test the filtrate for proteoses and peptones with Millon's reagent. Neutralize the main bulk of the filtrate with $Na_2CO_3$ and filter out the acid albuminate. Heat the filtrate to boiling. A precipitate here is due to coagulable proteins. Filter and evaporate the filtrate to 200 cc. on the water-bath. Saturate the solution with solid $(NH_4)_2SO_4$ and filter out the albumose precipitated. The precipitate of the albumose is complete only after long standing of a completely saturated solution.

Make tests for acid albuminate in precipitate and for peptone in filtrate.

(*b*) *Pancreatic digestion of egg white.*—Boil four eggs hard and rub the whites through a sieve. Put them in a stoppered flask with 500 cc. of 1 per cent $Na_2CO_3$ solution containing 2 or 3 grams of pancreatin (commercial). Use a little thymol or chloroform to prevent putrefaction. The mixture is digested at 40° C. for about 72 hours, then the content of the flask is made faintly acid with acetic acid and boiled. Filter and evaporate the filtrate to a thin sirup. Set in a cool place until crystals of tyrosin appear. Examine some of the crystals under the microscope. Decant through muslin (reserve filtrate for separation of leucin) and wash with water. These crystals may be used for the following experiments. (If crystals of leucin have separated also, heat material cautiously in a little water and filter. The leucin is dissolved.)

1. Put a few crystals of tyrosin in a test-tube with a little water, add a few drops of Millon's reagent, and

heat gently. The liquid, especially on standing, turns deep red.

2. *Piria's test* (*Hawk*).—"Warm a little tyrosin on a watch-glass on a boiling water-bath for 20 minutes with 3–5 drops of concentrated $H_2SO_4$. Tyrosin-sulphuric acid is formed in the process. Now add $CaCO_3$ in substance slowly, with stirring, until the reaction of the solution is no longer acid. Filter, concentrate the filtrate, and add to it a few drops (avoid an excess) of very dilute neutral ferric chloride. A purple or violet color, due to the formation of the ferric salt of tyrosin-sulphuric acid, is produced. This is one of the most satisfactory tests for the identification of tyrosin."

*Leucin.*—The solution decanted from the tyrosin is evaporated further on the water-bath, and on standing crystals of leucin form on the surface. Examine under the microscope. Compare with tyrosin. For further experiments see Hawk.

## DIGESTION OF FAT

EXPT. 87. *Digestion of fat (or action of pancreatin on fats).*—Free a fresh pancreas (pig or beef) from fat. Cut up with the scissors. Then grind a few grams with fine sand in a mortar to a paste. Add water until it is a creamy consistency. Melt some butter in a test-tube and let curd settle. Mix a few grams with an equal volume of pancreas paste. Add some chloroform water and keep at a temperature of 40° for several hours. Then test the reaction with rosolic acid. It is well to know the reaction of the mixture before it is put in the warm oven.

*"Litmus-Milk" test.*—Into each of two test-tubes introduce 10 cc. of milk and a small amount of litmus powder. To the contents of one tube add 3 cc. of *neutral* pancreatic extract, and to the contents of the other tube add 3 cc. of water or of *boiled* neutral pancreatic extract. Keep the tubes at 40° C. and note any changes which may occur. What is the result, and how do you explain it?

### QUESTIONS

1. In what forms are the carbohydrates of the food absorbed in the body?
2. What are the bad effects of an excessive use of sugar in the diet?
3. What is saccharine? What is its place in the diet?

4. Under what circumstances are sugar and starch avoided in the diet?

5. What protein foods would you use in the diet of children?

6. What objections are urged against a high protein diet? A low protein diet?

7. Name some foods that yield purin bodies.

8. What results are likely to follow an excessive use of fat in the diet?

9. In what pathological condition is fat particularly prescribed?

10. Why is cows' milk modified in infant feeding?

# APPENDIX

1. *Constant temperature bath for gelatinization of starch.*—Place about 15 cc. of a 1 per cent starch suspension in a test-tube inserted in a cork, which will serve as a stopper to the outside tube of an ordinary Beekmann boiling point apparatus. The outside tube may then be filled with any desired liquid of constant boiling point, and the inner tube suspended so as to be in contact with only the vapors of the boiling bath. The vapors are condensed and run back into the tube from a side arm connected with a condenser. Samples of the starch may be removed from the inner tube periodically and examined under the microscope. By using baths of various liquids boiling below 100° C., the effect of long-continued but low heat on starch granules may be easily observed.

2. *Egg albumin.*—Cut up with scissors the whites of one dozen eggs, turn these into an equal volume of saturated $(NH_4)_2SO_4$, then filter (the precipitate is globulin). To the filtrate add acetic acid (10 per cent) until a permanent precipitate appears, then add 1 cc. acetic acid for each 100 cc. of solution. Stand over night; filter. Dry the precipitate and powder.

3. *Globulin.*—Dissolve the globulin precipitate by adding water (the ammonium sulphate in the precipitate is sufficient to make a dilute salt solution). Reprecipitate by adding about an equal volume of alcohol. Filter, dry, powder.

4. *Ovovitellin.*—Shake the yolks of two eggs with 200 cc. of ether and add 5 cc. of alcohol. Let the precipitate settle and pour off the ether solution as completely as possible, then add 100 cc. of a 15 per cent NaCl solution to the precipitate. On shaking, the precipitate dissolves in the salt solution, which is somewhat turbid. Put the liquid in a separatory funnel and shake it with an equal volume of ether. Separate the aqueous fluid from the ether and let it stand until the next day. If it is turbid shake it again with ether. Draw off the aqueous fluid again, measure it, and pour into it ten times its volume of water. Let this stand until next day and filter. Wash with water and then with alcohol. This precipitate is mixed with some lecithin. This may be removed by adding absolute alcohol to the precipitate and boiling in a flask on the water-bath. Filter, wash with alcohol, then with ether, then grind in a mortar and dry in a desiccator. A fine yellow powder is different from that of the first precipitate (see "A Laboratory Manual of Physiological Chemistry," by Salkowski, 1904).

5. *Ovomucoid.*—Add to whites of six eggs four times their volume of water, shake thoroughly, and pour into twice the volume of boiling water made very slightly acid by acetic acid. Boil briskly over free flame (granite pan) to thoroughly coagulate albumin and globulin. Filter and evaporate filtrate (which should give no precipitate with mercuric chloride) on water-bath to about 40 cc., pour into 100 cc. absolute alcohol, let stand until precipitate settles. Filter, wash with alcohol and ether, and dry.

(*a*) *Hydrolysis of ovomucoid.*—To a small portion of the ovomucoid add 10 cc. dilute HCl (10 per cent) and boil for five minutes, adding water as necessary. Add KOH to neutralize, and test the resulting solution with Fehling's reagent for reducing substances. If a precipitate does not appear at once allow the solution to stand until it becomes cool. If this method does not give satisfactory proof of a reducing substance, long-continued boiling with HCl as directed in the hydrolysis of mucin may be employed.

According to Salkowski, nucleo-protein may be obtained as follows:

6. Free the pancreas from fat. Heat 200 grams of finely chopped pancreas to boiling with 1 liter of water, keep boiling for ten minutes, filter, and add cautiously to the filtrate, while still warm, about 10–15 cc. of 30 per cent acetic acid, until a fine, flocculent precipitate begins to settle. If the precipitate does not settle well it is advisable to heat again. Filter, wash with water, remove the precipitate from the filter, grind it with 50 cc. of absolute alcohol, filter, treat the precipitate with about 50 cc. of ether, filter next day, wash once with ether, and grind. The presence of phosphorus and pentose may be very easily shown in the somewhat impure nucleo-protein thus obtained.

(*a*) To detect the phosphorus fuse a small quantity with the oxidizing mixture, and then proceed as under Casein.

(*b*) To detect the pentose the phloroglucin and the orcin tests may be used.

*Phloroglucin test.*—Pour on a very small quantity of the substance in a few cubic centimeters of hydrochloric acid, add a little phloroglucin, and heat to boiling—cherry-red color, then turbidity. Let cool somewhat, shake with an equal volume of amyl alcohol, and examine this with the spectroscope—absorption band between *D* and *E*.

*Orcin test.*—Instead of phloroglucin take a few orcin crystals and proceed in the same way—reddish blue color, then precipitation of a blue pigment. The amyl alcohol turns red and after some time emerald green. Examine with the spectroscope—absorption band between *C* and *D*.

*7. Separation of proteose and peptones according to Hawk.*—"Place 50 cc. of proteose-peptone solution in an evaporating dish or casserole, and *half saturate* it with $(NH_4)_2SO_4$ solution, which may be accomplished by adding an equal volume of *saturated* $(NH_4)_2SO_4$ solution. At this point note the appearance of a precipitate of the *primary proteoses* (protoproteose and heteroproteose). Now heat the half-saturated solution and its suspended precipitate to boiling, and *saturate* the solution with *solid* $(NH_4)_2SO_4$. At full saturation the *secondary proteoses* (deuteroproteoses) are precipitated. The peptones remain in solution.

"Proceed as follows with the precipitate of proteoses: Collect the sticky precipitate on a rubber-tipped stirring rod, or remove it by means of a watch-glass to a small evaporating dish, and dissolve it in a little water. To remove the $(NH_4)_2SO_4$, which adhered to the precipitate

and is now in solution, add $BaCO_3$, boil, and filter off the precipitate of $BaSO_4$. Concentrate the proteose solution to a small volume and make the following tests:

"(*a*) *Biuret test.*

"(*b*) *Precipitation by* $HNO_3$.—What would a precipitate at this point indicate?

"(*c*) *Precipitation by trichloracetic acid.*—This precipitate dissolves on heating and returns on cooling.

"(*d*) *Precipitation by picric acid.*—This precipitate also disappears on heating and returns on cooling.

"(*e*) *Precipitation by potassio-mercuric iodide* and HCl.

"(*f*) *Coagulation test.*—Boil a little in a test-tube. Does it coagulate?

"(*g*) *Acetic acid and potassium ferrocyanide test.*

"The solution containing the peptones should be cooled and filtered, and the $(NH_4)_2SO_4$ in solution removed by boiling with $BaCO_3$. After filtering off the $BaSO_4$ precipitate, concentrate the peptone filtrate to a small volume and repeat the tests as given under the proteose solution. In the biuret test the solution should be made very strongly alkaline with *solid* KOH."

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